Susceptibility to Infections and Immune Status in Inuit Infants Exposed to Organochlorines

Éric Dewailly,[1,2] Pierre Ayotte,[1,2] Suzanne Bruneau,[1] Suzanne Gingras,[1] Marthe Belles-Isles,[3] and Raynald Roy[3]

[1]Unité de Recherche en Santé Publique, Centre Hospitalier Universitaire de Québec, Pavillon CHUL, Beauport, Québec, Canada; [2]Département de Médecine Sociale et Préventive, Université Laval, Sainte-Foy, Québec, Canada; [3]Unité de Recherche en Rhumatologie et Immunologie, Centre Hospitalier Universitaire de Québec, Pavillon CHUL, Sainte-Foy, Québec, Canada

We investigated whether organochlorine exposure is associated with the incidence of infectious diseases in Inuit infants from Nunavik (Arctic Quebec, Canada). We compiled the number of infectious disease episodes during the first year of life for 98 breast-fed and 73 bottle-fed infants. Concentrations of organochlorines were measured in early breast milk samples and used as surrogates to prenatal exposure levels. Immune system parameters were determined in venous blood samples collected from infants at 3, 7, and 12 months of age. Otitis media was the most frequent disease, with 80.0% of breast-fed and 81.3% of bottle-fed infants experiencing at least one episode during the first year of life. During the second follow-up period, the risk of otitis media increased with prenatal exposure to *p,p′*-DDE, hexachlorobenzene, and dieldrin. The relative risk (RR) for 4- to 7-month-old infants in the highest tertile of *p,p′*-DDE exposure as compared to infants in the lowest tertile was 1.87 [95% confidence interval (CI), 1.07–3.26]. The RR of otitis media over the entire first year of life also increased with prenatal exposure to *p,p′*-DDE (RR, 1.52; CI, 1.05–2.22) and hexachlorobenzene (RR, 1.49; CI, 1.10–2.03). Furthermore, the RR of recurrent otitis media (≥ 3 episodes) increased with prenatal exposure to these compounds. No clinically relevant differences were noted between breast-fed and bottle-fed infants with regard to immunologic parameters, and prenatal organochlorine exposure was not associated with immunologic parameters. We conclude that prenatal organochlorine exposure could be a risk factor for acute otitis media in Inuit infants. *Key words*: breast-feeding, insecticides, Inuit, lymphocytes, organochlorines, otitis, polychlorinated biphenyls. *Environ Health Perspect* 108:205–211 (2000). [Online 20 January 2000]
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The contamination of the arctic aquatic food chain by organochlorine compounds (OCs) has been documented during the last decade (*1–4*). This group of chemicals includes pesticides (e.g., dieldrin, mirex, and toxaphene), industrial compounds and by-products of various industrial processes [e.g., hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs), polychlorodibenzo-*p*-dioxins (PCDDs), and polychlorodibenzofurans (PCDFs)]. In spite of regulatory actions adopted since the late 1970s in North America and Western Europe to limit their emission into the environment, these substances are still being released because of improper storage and disposal and because of their ongoing use in other parts of the world.

Once emitted into the environment at the middle and lower latitudes, OCs reach the Arctic via long-range atmospheric and oceanic transport (*1*). High lipophilicity and poor biodegradability lead to their bioconcentration in the fatty tissues of organisms. Biomagnification also occurs through the arctic aquatic food chain, resulting in relatively high levels of contaminants in top predator species (polar bear, beluga, seal) (*2–5*). For cultural and economic reasons, the Inuit from Nunavik (Arctic Quebec, Canada) rely heavily on marine foods for their subsistence. Their large consumption rate of sea mammal fat (in particular ringed seal and beluga) leads to body burdens of various OCs that exceed those of southern Quebec populations by factors varying from 2 to 10 (*6–10*).

Several OCs display immunotoxic properties in both laboratory animals and humans. The most potent OCs are substances structurally related to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), such as non- and mono-*ortho* chloro-substituted PCBs and 2,3,7,8-chloro-substituted PCDD/PCDFs. These molecules can bind to the aryl hydrocarbon receptor (*11*), and the ligand–receptor complex triggers the expression of genes that are involved in cell proliferation and differentiation (*12*). In almost all of the animal species tested, including primates, PCDD/PCDFs and PCBs produce myelosuppression, immunosuppression, thymic atrophy, and inhibition of immune complement system components (*13*). Exposure to TCDD during pre- and/or postnatal life results in more severe effects than if the chemical is administered during adult life and in some species may be a prerequisite for immunosuppression (*14–15*). In fact, available evidence in laboratory animals suggests that the maturation of the immune system is especially vulnerable to the adverse effects of dioxin-like compounds, chlordane, hexachlorobenzene, polycyclic aromatic hydrocarbons, and possibly endocrine-disrupting compounds such as DDT and kepone (*16,17*).

In children and young adults accidentally exposed to PCBs and PCDFs in Taiwan (Yu-Cheng disease), serum IgA and IgM concentrations as well as percentages of total T cells, cytotoxic T cells, and suppressor T cells were decreased as compared to values in age- and sex-matched controls (*18*). The investigation of delayed-type hypersensitivity responses further indicated that cell-mediated immune system dysfunction was more frequent among patients than controls. Infants born to mothers who had Yu-Cheng disease had more episodes of bronchitis or pneumonia during their first 6 months of life than did unexposed infants from the same neighborhoods (*19*). The authors speculated that the increased frequency of pulmonary diseases could be the result of a generalized immune disorder induced by transplacental or breast milk exposure to dioxin-like compounds, more likely PCDFs (*19*). Children 8 to 14 years of age who were born to mothers with Yu-Cheng disease are more prone to middle ear diseases than matched controls (*20*).

For many years young native children from Nunavik have had a high incidence of infectious diseases, in particular meningitis, bronchopulmonary, and middle ear infections (*21–23*). Otitis media and the damage it can

Address correspondence to É. Dewailly, Unité de Recherche en Santé Publique, Centre Hospitalier Universitaire de Québec (Pavillon CHUL), 2400 d'Estimauville, Beauport, Québec, Canada G1E 7G9. Telephone: (418) 666-7000, ext. 245. Fax: (418) 666-2776. E-mail: edewailly@cspq.qc.ca

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cause to hearing is a major problem for Inuit children and adults. In fact, Inuit in Nunavik report hearing loss as their most common chronic health problem (*24*). In 1984, 78% of Inuit school children in Kuujjuarapik had current or previous ear infections and 23% of the children had a significant hearing loss in one or both ears (*25*). The prevalence of hearing loss among 74 students tested in Inukjuak in 1988 was 24% (*23*). In 1995–1996, screenings performed on the coast of Ungava found a 28% rate of hearing loss in students (*26*). The reason for the high incidence of middle ear infections in Inuit children is unknown. Genetically determined IgG2 deficiency may be involved (*27*), as well as anatomical or physiologic factors (such as the morphology of the Eustachian tube), dietary habits, housing (crowding, humidity level), community sanitation, and personal hygiene (*22*).

Because of the immunotoxic properties displayed by some OCs, in particular after perinatal exposure, we hypothesized that part of the high infection incidence among Inuit infants could be related to the relatively high maternal body burden of these contaminants. To test this hypothesis, we organized a cohort study in Arctic Quebec to investigate the relationship between OC exposure, the immune status, and the occurrence of infectious diseases among Inuit infants during their first year of life.

Methods

Subjects. Approximately 7,000 Inuit people live in Nunavik; 14 communities are scattered along 1,500 km of seashore. Nunavik is located between 55 and 63° north latitude (Figure 1). All Inuit women who gave birth between July 1989 and September 1990 at the two regional hospitals located in Puvirnituq (East Hudson Bay) and Kuujjuaq (Ungava Bay) were asked to participate in the study. This represented approximately 83% of all pregnancies in Nunavik; at-risk pregnancies (for maternal or fetal reasons) were transferred to hospitals located in Montreal, Quebec, Canada. Informed consent was obtained from all women who entered the study. The protocol was approved by the medical ethical committee of the Centre Hospitalier Universitaire de Québec (Pavillon CHUL, Sainte-Foy, Quebéc, Canada). A pretested maternal questionnaire was administered at the infant's birth by a bilingual interviewer (English/Inuktitut) to obtain information regarding the community of residence, maternal body weight after delivery, pregnancy duration, and breast-feeding history. The sex and weight of the newborn were obtained from the medical files.

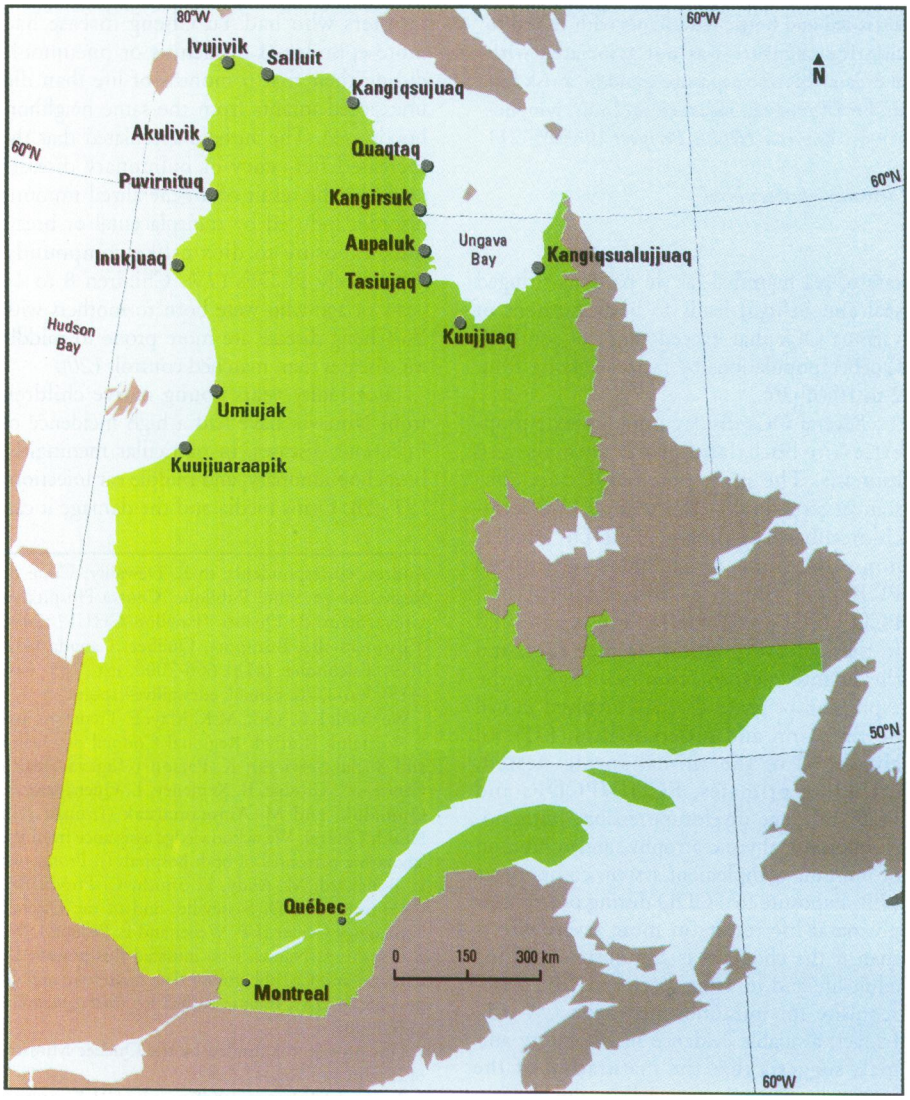

Figure 1. Location of Nunavik (Arctic Quebec, Canada).

Measures of OC exposure. We used OC concentrations in breast milk fat as an index of *in utero* exposure to these substances. Organochlorine concentration in breast milk fat is a good surrogate of maternal body burden (*28*). These compounds cross the placental barrier to reach the developing fetus; concentrations of various OCs in milk fat, maternal blood lipids, and cord blood lipids are highly correlated (*28*). Therefore, OC concentration in fat from any of these biologic fluids adequately reflects prenatal exposure to these substances.

Prenatal OC exposure was determined only in the breast-fed group, but bottle-fed infants were also prenatally exposed to these compounds. In contrast to breast-fed infants who were exposed to OCs through maternal milk consumption, postnatal exposure of bottle-fed infants to organochlorines before the introduction of solid foods can be considered negligible because of the low levels of these substances normally found in evaporated milk and cow's milk [i.e., < 0.5 µg PCB/kg according to the 1985–1988 Total Diet Program (*29*)] as compared to those found in the breast milk of Inuit women (averaging approximately 100 µg PCB/kg) (*7*). The volume of blood drawn from infants at follow-up visits was insufficient to allow OC determination and therefore a direct measure of postnatal exposure was not available.

Breast milk sampling and OC determination. Immature breast milk samples (40 mL) were collected in polycarbonate vials within 3 days after delivery. The sampling duration did not exceed 24 hr. Milk samples were frozen at -20°C and sent to the Quebec Toxicology Center laboratory (Sainte-Foy, Quebéc, Canada) for organochlorine analysis. Concentrations of 10 PCB congeners [International Union of Pure and Applied Chemistry (IUPAC) numbers 28, 52, 101, 118, 138, 153, 170, 180, 183, and 187] and 8 chlorinated pesticides or metabolites (*p,p'*-DDE, mirex, heptachlor epoxide, α- and γ-chlordane, hexachlorobenzene, endrin, and

dieldrin) were measured in milk fat extracts using high resolution gas chromatography with electron capture detection, according to the method previously described by Dewailly et al. (*8*).

Follow-up of infants. At 3, 7, and 12 months of age, infants were seen by a nurse at the nursing station in their community. At these visits the nurse performed blood sampling and a medical evaluation. Information was obtained regarding the breast-feeding status as well as the time of introduction and the identity of breast milk substitutes and solid foods. The nurse was also trained to screen for the skin problems that were observed in Taiwanese children after prenatal and postnatal exposure to PCBs and PCDFs (*19*). These skin problems were scalp folliculitis, blackhead or acne on the face or body, enlargement of moebius glands and eyelid edema, toenail deformations (concave, ingrown nail), and brown hyperpigmentation of the lips, gums, nails, and the surrounding skin.

The occurrence of selected infectious diseases of high prevalence in Nunavik was documented during the first year of life (from birth to 3 months, between 4 and 7 months, and from 8 to 12 months of age). More specifically, the occurrence of the following infections was recorded: otitis media (ear infection), tonsillitis and pharyngitis, laryngitis and tracheitis, bronchitis and bronchiolitis, pneumonia, flu, sinusitis and rhinitis, and various skin problems (impetigo, cellulitis, allergies, and eczema). Otitis media was the main focus of the present study. A specific program on otitis was introduced in 1984 to improve diagnosis, treatment, and rehabilitation in Inuit from Nunavik. Infants with fever, irritability, pain accompanied or not accompanied by discharge from the ear were tentatively identified as having otitis media. Diagnosis was further supported by abnormal tympanic membrane appearance (redness, bulging, swelling) on otoscopic examination. If the same health problem occurred more than once, a minimal number of weeks had to elapse before it was considered a new episode rather than a continuation of the same episode. For example, an otitis episode was deemed new if it occurred ≥ 3 weeks after the previous one.

Blood sampling and immunologic parameters. Blood samples (~ 2 mL) were collected from babies by nurses at 3, 7, and 12 months of age. For cellular analyses, samples were immediately processed. Serum samples were stored at -20°C and sent to the immunology laboratory at the Centre Hospitalier Universitaire de Québec for immunoglobulin determination. Lymphocyte counts were determined by whole-blood fluorescence cell sorter analysis combined with the quantification of white blood cells by a cell counter. Lymphocyte subsets were determined by flow cytofluorometry analysis with direct staining of the whole blood cells performed onsite, using monoclonal antibodies labeled with fluorescein isothiocyanate (Profile; Beckman Coulter, Fullerton, CA). We evaluated CD3 (T cells), CD4 (helper T cells), CD8 (cytotoxic T cells), and CD20 (B cells). Briefly, we diluted 50 µL peripheral blood in 50 µL PBS containing the appropriate amounts of monoclonal antibodies and incubated the solution for 15 min at 4°C. After a wash step, the red cells were lysed (Immunolyse solution; Beckman Coulter), a cell fixative was added, the solution was washed twice, and the stained portion of the white blood cells was sent to the immunology laboratory to be measured on a cytofluorometer (Profile). Immunoglobulins (IgG, IgA, and IgM) were measured by nephelometry (Dade Behring, Deerfield, IL).

Statistical analyses. A continuous variable expressed the number of episodes of a health problem since the last visit to the nursing station. A dichotomized variable was also created with two categories: no episode during the period, and one episode or more. We performed additional analyses using two categories: no episode during the first year of life, and three or more episodes during the first year of life. Various health problems were also grouped in large classes: ear, nose, and throat diseases; pulmonary diseases; and skin diseases.

We used Student's *t*-test or the chi-square test to evaluate the statistical significance of differences between breast-fed and bottle-fed groups with regard to selected participant characteristics. We computed relative risks for breast-fed infants for the occurrence of infectious diseases during each follow-up period as compared to the bottle-fed group. Relative risks were also used to assess differences in disease occurrence between breast-fed subgroups (tertiles of OC exposure using the lowest tertile as the comparison group). We conducted logistic regression analyses to control for confounding factors, and we assessed differences between bottle-fed and breast-fed groups for immunologic parameters using Student's *t*-tests.

OC concentrations in breast milk displayed log-normal distributions; therefore, we used log-transformed values in the statistical analyses. We tested associations between OC exposure and immunologic parameters with Pearson correlation coefficients. We included these OCs as independent variables in statistical analyses: the sum of the three most abundant PCB congeners found in breast milk (IUPAC numbers 138, 153, and 180), and those chlorinated pesticides that were detected in > 85% of breast milk samples (DDE, dieldrin, HCB, and mirex). We assigned a value equal to half of the detection limit of the analytical method to those results categorized "not detected."

Results

Among the 222 women who delivered in one of the two regional hospitals, 213 agreed to participate and were enrolled in the study (95.9% participation rate). Information on health problems for at least one follow-up period during the first year of life was available for 171 infants (77.0%). Fifteen (8.8%) infants were seen only once (either at the first, second, or third follow-up visit), 38 (22.2%) were seen twice, and 118 (69.0%) attended all three follow-up visits. Major reasons for not attending a follow-up visit included being outside the community (for example, moving to campground during the hunting season) and fear of venous puncture for the infant. There was no statistically significant difference between newborns who attended one or more visits and those who did not attend any follow-up visits with regard to gestation length, birth weight, and maternal age and weight (data not shown).

Of the 171 participating newborns, 98 (57.3%) were breast-fed and 73 (42.7%) bottle-fed. Organochlorine concentrations were determined in breast milk fat; therefore, data were only available for breast-feeding mothers; 94 mothers provided enough milk to allow OC analysis. Table 1 presents various characteristics of mothers enrolled in this study and their newborns, according to breast-feeding status. We obtained maternal characteristics by questionnaire; several Inuit women were not able to provide precise information for cultural reasons, most notably on their weight. Analysis of available data revealed that mothers who breast-fed their newborns had similar weight and gestation length as compared to those who bottle-fed, but were significantly older (by 2.7 years on average). Newborns from both groups did not differ in terms of sex ratio and weight.

Table 2 contains mean concentrations of selected organochlorine and the corresponding tertile limits. *p,p'*-DDE showed the highest concentration, followed by PCBs (the sum of congeners 138, 153, and 180), HCB, dieldrin, and mirex (Table 1). A more complete description of the analytical results was previously published (*8*).

Acute otitis media was the most frequent health problem among Inuit newborns during the first year of life, with 80.5% of all infants experiencing one or more episodes (80.0% of ever breast-fed and 81.3% of never breast-fed infants). Of the 118 infants for which follow-up was complete, 23 (20%), 18 (15%), 28 (24%), 19 (16%), 19 (16%), and 11 (9%) had 0, 1, 2, 3, 4, and ≥ 5 otitis episodes, respectively. The percentage of ever

breast-fed infants who contracted ≥ 3 episodes of otitis media during the first year of life (40.5%) was similar to that of never breast-fed (bottle-fed) infants (44.7%). During the first year of life, ever breast-fed and never breast-fed infants experienced a similar number of episodes with acute otitis media {mean = 2.3, [95% confidence interval (CI), 1.8–2.7] vs. 2.7 (CI, 2.1–3.2); p = 0.21}.

The second most frequent health problem was pulmonary infections: 59.3% (54.3% of ever breast-fed and 66.7% of never breast-fed infants). Ever breast-fed infants contracted fewer pulmonary infections than bottle-fed infants [mean = 1.2, (CI, 0.9–1.6) vs. 2.0 (CI, 1.3–2.6); p = 0.05]. None of the skin problems reported in Taiwanese children born to mothers with Yu-Cheng disease were observed in Inuit infants.

The relative risk of various infectious diseases among breast-fed infants during the first year of life is presented in Table 3. During the first follow-up period, the risk of otitis media among breast-fed infants appeared lower than in bottle-fed infants, although statistical significance was not reached. The relative risks of otitis media later during the first year of life were close to 1. The relative risk of pneumonia during the second follow-up period was lower in breastfed infants as compared to the bottle-fed group. The incidence of pharyngitis and tonsillitis among bottle-fed infants during the third follow-up period was 10.9%, although no breast-fed infant contracted this infectious disease.

Table 4 presents the relative risks of otitis media among breast-fed infants according to tertiles of organochlorine concentrations in breast milk (reflecting prenatal exposure). Most notably, during the second period, the relative risks of acute otitis media increased for infants in the third tertiles of OC concentrations in breast milk fat (HCB, DDE, and dieldrin). Among the 65 breast-fed infants who were seen at each follow-up visit during the first year of life, the incidence of otitis during the entire first year of life increased with tertiles of prenatal OC exposure (HCB, DDE; Table 5). Prenatal exposure to HCB and DDE also increased the risk of contracting ≥ 3 otitis media episodes (Table 6). The mean breast-feeding duration of infants enrolled in the present study was 35 weeks, ranging from 4 to 52 weeks. Logistic regression analysis taking into account breast-feeding duration, maternal age, and the duration of past breast-feeding episodes yielded similar associations. OC exposure was not related to the incidence of bronchopulmonary diseases (data not shown).

Table 7 presents results for the determination of various immunologic parameters in peripheral blood samples from breast-fed and bottle-fed infants on three visits during the first year of life. At 3 months of age, concentrations of white blood cells and lymphocytes (more specifically those of the CD4 subtype) were lower ($p \leq 0.05$) in blood samples from breast-fed babies as compared to those from the bottle-fed group. At 7 and 12 months of age, IgA concentrations were lower ($p \leq 0.05$) in breast-fed infants than in bottle-fed infants, as were

Table 1. Characteristics of the study population according to breast-feeding status.

Characteristics	Breast-feeding, n = 98 No.	Mean (CI)	No breast-feeding, n = 73 No.	Mean (CI)	p-Value
Mothers					
Age (years)	87	24.2 (23.2–25.3)	65	21.5 (20.3–22.8)	0.002
Weight (kilograms)	56	59.7 (57.4–61.9)	55	59.0 (57.3–60.7)	0.63
Gestation length (weeks)	86	38.9 (38.5–39.3)	63	39.1 (38.7–39.5)	0.48
Newborns					
Sex (male, %)	98	45.9%	73	54.8%	0.25
Weight (kilograms)	87	3.47 (3.38–3.57)	66	3.47 (3.36–3.58)	0.99

Table 2. Concentrations of OC in breast milk fat (microgram per kilogram).

Compound	No.	GM (CI)	T1[a]	T2	T3
ΣPCBs[b]	94	621 (530–727)	< 432	432–873	> 873
HCB	94	107 (92–125)	< 86	86–146	> 146
DDE	94	962 (835–1,108)	< 730	730–1,320	> 1,320
Dieldrin	94	30 (26–35)	< 21	21–43	> 43
Mirex	94	14 (12–16)	< 9	9–18	> 18

GM, geometric mean.
[a]Tertile limits. [b]Sum of congeners 138, 153, and 180.

Table 3. Relative risks (RRs) of various health problems in breast-fed Inuit infants during the first year of life, as compared to bottle-fed Inuit infants.

Health problems	0–3 month follow-up period Bottle-fed, (n = 65), %[a]	Breast-fed, (n = 90), RR (CI)	4–7 month follow-up period Bottle-fed, (n = 68), %	Breast-fed, (n = 90), RR (CI)	8–12 month follow-up period Bottle-fed, (n = 55), %	Breast-fed, (n = 78), RR (CI)
Ear, nose, throat, eyes	56.9	1.00 (0.75–1.32)	70.6	0.94 (0.76–1.17)	56.4	0.98 (0.72–1.33)
Acute otitis media	47.7	0.70 (0.47–1.03)	58.8	0.96 (0.74–1.26)	45.5	1.19 (0.83–1.69)
Pharyngitis, tonsillitis	1.5	5.78 (0.74–45.07)	8.8	0.88 (0.31–2.50)	10.9	0 (–)
Flu	12.3	1.99 (0.94–4.18)	11.8	0.85 (0.35–2.09)	7.3	0.35 (0.07–1.86)
Conjunctivitis	9.2	1.93 (0.80–4.65)	5.9	0.76 (0.20–2.91)	7.3	0.35 (0.07–1.86)
Pulmonary diseases	26.2	1.15 (0.69–1.92)	33.8	0.89 (0.56–1.40)	30.9	0.66 (0.37–1.20)
Bronchitis, broncheolitis	18.5	1.14 (0.60–2.19)	23.5	1.04 (0.59–1.82)	21.8	0.53 (0.24–1.17)
Pneumonia	6.2	2.35 (0.80–6.87)	22.1	0.25 (0.10–0.66)	11.3	0.69 (0.24–2.02)
Skin affections	18.5	1.20 (0.63–2.28)	22.1	0.86 (0.46–1.59)	16.4	0.86 (0.38–1.94)

[a]Percentage of infants with at least one episode of the infectious disease during the follow-up period.

Table 4. Relative risk (RR) of acute otitis media among breast-fed Inuit infants during three periods in the first year of life, according to prenatal exposure to selected OCs (in tertiles T1, T2, and T3[a]).

	0–3 month follow-up period T1 (n = 27) %[b]	T2 (n = 29) RR (CI)	T3 (n = 27) RR (CI)	4–7 month follow-up period T1 (n = 26) %	T2 (n = 28) RR (CI)	T3 (n = 28) RR (CI)	8–12 month follow-up period T1 (n = 24) %	T2 (n = 23) RR (CI)	T3 (n = 25) RR (CI)
ΣPCBs[c]	29.6	1.28 (0.61–2.70)	0.88 (0.37–2.07)	46.2	1.16 (0.68–1.99)	1.47 (0.90–2.39)	50.0	1.39 (0.86–2.26)	0.88 (0.49–1.60)
HCB	25.8	0.81 (0.30–2.16)	1.80 (0.88–3.69)	46.7	1.03 (0.58–1.82)	1.55 (1.00–2.42)	46.4	1.29 (0.76–2.21)	1.26 (0.75–2.12)
DDE	26.7	1.47 (0.70–3.12)	1.05 (0.44–2.49)	37.0	1.68 (0.95–2.96)	1.87 (1.07–3.26)	46.2	1.63 (1.01–2.61)	0.89 (0.46–1.70)
Dieldrin	28.6	0.74 (0.31–1.79)	1.75 (0.85–3.59)	42.3	1.29 (0.75–2.23)	1.75 (1.05–2.91)	64.0	0.69 (0.40–1.17)	0.85 (0.53–1.38)
Mirex	26.7	1.20 (0.53–2.74)	1.34 (0.62– 2.90)	40.0	1.88 (1.14–3.08)	1.43 (0.83– 2.46)	54.2	1.00 (0.59–1.68)	1.00 (0.59–1.68)

[a]Tertile limits are defined in Table 2. [b]Percentage of infants with at least one episode of the infectious disease during the follow-up period. [c]Sum of congeners 138, 153, and 180.

CD4/CD8 ratios, although the differences did not reach statistical significance. None of the immunologic parameters were associated with prenatal OC exposure.

Discussion

Inuit children from various regions of the Arctic are at increased risk for otitis media (*25,30,31*). In the present study, 80.5% of Inuit infants experienced at least one otitis media episode during the first year of life. Ingvarsson and colleagues (*32*) reported an annual incidence of 38% for boys and 30% for girls in Sweden. Among children in Finland, the annual incidence of otitis media was 38 and 28% for boys and girls, respectively (*33*), and in one American study conducted in Boston, it was 62% for boys and girls combined (*34*).

Infante-Rivard and Fernandez (*35*) reviewed the literature on otitis media in children and noted that breast-feeding duration appears to be a protective factor, especially very early in life when children are more susceptible to the disease and more likely to have recurrent and persistent otitis media with effusion. In Labrador, Canada, a study among 238 Inuit revealed that breast-feeding seems to offer some protection against middle ear infection (*36*). No children who were bottle-fed after 6 months of age had otitis media (0/21), as compared to 67 among the 160 who were bottle-fed as newborns. In Nunavik (Arctic Quebec), Dufour (*37*) observed that protection through breast-feeding seems to last only for the first 3 months, after which the relationship does not hold and may even be reversed.

Results from the present study also suggest that breast-feeding during the first trimester affords protection against acute otitis media, although this result did not reach statistical significance (Table 3). The protection early in life could be related to the transfer of maternal antibodies from the mother to the infant during the first months of breast-feeding. Organochlorines accumulate in the newborn during the breast-feeding period (*38–40*) and could eventually reach concentrations that are detrimental to immune system function. However, we could not address the effect of postnatal OC exposure in the present study. The limited amount of blood collected from infants at follow-up visits was not enough to allow the determination of organochlorine concentrations.

Table 5. Relative risk (RR) of experiencing at least one episode of acute otitis media among breast-fed Inuit infants during the first year of life, according to the prenatal exposure to selected OCs (in tertiles T1, T2, and T3[a]).

OC	T1 (*n* = 22) %[b]	T2 (*n* = 21) RR (CI)	T3 (*n* = 22) RR (CI)
ΣPCBs[c]	68.2	1.25 (0.89–1.75)	1.28 (0.92–1.77)
HCB	64.0	1.30 (0.91–1.87)	1.49 (1.10–2.03)
DDE	59.1	1.55 (1.07–2.24)	1.52 (1.05–2.22)
Dieldrin	71.4	1.11 (0.79–1.56)	1.26 (0.93–1.71)
Mirex	66.7	1.28 (0.91–1.79)	1.36 (0.99–1.86)

[a]Tertile limits are defined in Table 2. [b]Percentage of 22 breast-fed infants in tertile 1 for which follow-up was complete, with at least one episode of acute otitis media during the first year of life. [c]Sum of congeners 138, 153, and 180.

Table 6. Relative risk (RR) of experiencing three or more episodes of acute otitis media among breast-fed Inuit infants during the first year of life, according to the prenatal exposure to selected OCs (in tertiles T1, T2, and T3[a]).

OC	T1 (*n* = 22) %[b]	T2 (*n* = 21) RR (CI)	T3 (*n* = 22) RR (CI)
ΣPCBs[c]	31.8	1.56 (0.48–5.60)	1.65 (0.49–5.57)
HCB	28.0	1.07 (0.28–4.18)	3.71 (1.10–12.56)
DDE	19.1	4.64 (1.19–18.11)	3.48 (0.86–14.11)
Dieldrin	30.0	0.96 (0.26–3.53)	3.50 (0.95–12.97)
Mirex	37.5	1.21 (0.35–4.15)	1.03 (0.31–3.43)

[a]Tertile limits are defined in Table 2. [b]Percentage of 22 breast-fed infants in tertile 1 for which follow-up was complete, with three or more episodes of acute otitis media during the first year of life. [c]Sum of congeners 138, 153, and 180.

The relative risk of acute otitis media was related to prenatal exposure to some organochlorines during the second follow-up period (Table 4) as well as during the entire first year of life (Table 5). Furthermore, the risk of experiencing several episodes of the disease during the first year of life was also associated with prenatal exposure to these compounds (Table 6). Our results do not allow us to identify which compounds could be responsible for the increased susceptibility of Inuit infants to otitis media. Associations with otitis media were most consistent with *p,p′*-DDE and HCB. However, because all OCs originate from the same few food items and share a number of properties (persistence, liposolubility), their concentrations in breast milk are correlated to each other. Therefore, associations of health outcomes with *p,p′*-DDE or HCB may be due to other OCs found in the organochlorine mixture.

To our knowledge, this study is the first to report an association between the incidence of otitis media and chronic environmental exposure to organochlorines. In Taiwan, infants of mothers with Yu-Cheng disease, who were transplacentally exposed to PCBs and PCDFs, experienced a greater number of bronchitis episodes during their first year of life than unexposed children (*19*). Between 8 and 14 years of age, these children also had a significantly higher prevalence of middle ear diseases than their age-matched controls (*20*). In contrast, Weisglas-Kuperus et al. (*41*) observed no significant correlation between the number of periods with rhinitis, bronchitis, tonsillitis, and otitis during the first 18 months of life and either prenatal or postnatal exposure to PCB or dioxin-like compounds. The Weisglas-Kuperus et al. (*41*) study, which was conducted during 1990–1992 among 105 breast-fed infants from the Rotterdam area, did not report the incidence of otitis media during the first year of life. In our

Table 7. Immunologic parameters for breast-fed and bottle-fed infants during the first year of life.

	3-month-old infant				7-month-old infant				12-month-old infant			
	Bottle-fed		Breast-fed		Bottle-fed		Breast-fed		Bottle-fed		Breast-fed	
Immunologic parameters	No.[a]	Mean ± SD	No.	Mean ± SD	No.	Mean ± SD	No.	Mean ± SD	No.	Mean ± SD	No.	Mean ± SD
White blood cells (× 10^9/L)	47	13.3 ± 4.6	60	11.5 ± 3.9*	53	12.1 ± 3.5	66	13.0 ± 4.0	54	12.4 ± 5.0	68	12.5 ± 6.1
Lymphocytes (%)	48	62 ± 9	61	63 ± 10	54	61 ± 12	66	60 ± 9	54	55 ± 13	69	58 ± 10
Lymphocyte subsets (× 10^9/L)												
Lymphocytes	46	8.3 ± 3.1	59	7.3 ± 2.7	52	7.2 ± 2.5	68	7.8 ± 2.6	51	6.8 ± 2.9	68	7.2 ± 3.6
CD20	42	1.1 ± 0.7	53	0.9 ± 0.5	49	1.1 ± 0.5	62	1.2 ± 0.7	48	1.1 ± 0.6	65	1.2 ± 1.1
CD3	43	5.1 ± 2.0	53	4.5 ± 1.8	47	4.5 ± 1.8	60	4.9 ± 1.9	46	4.4 ± 2.0	63	4.4 ± 2.1
CD4	42	3.5 ± 1.4	52	2.9 ± 1.1*	46	2.9 ± 1.2	60	3.0 ± 1.2	46	2.5 ± 1.1	64	2.5 ± 1.1
CD8	42	1.5 ± 0.8	52	1.5 ± 0.8	45	1.5 ± 0.8	60	1.7 ± 0.8	46	1.7 ± 1.0	63	1.8 ± 1.2
CD4/CD8 ratio	42	2.6 ± 1.0	52	2.4 ± 1.2	45	2.2 ± 1.2	60	1.8 ± 0.7	46	1.9 ± 1.0	63	1.6 ± 0.5
Immunoglobulins (g/L)												
IgG	46	6.3 ± 2.2	63	6.4 ± 2.5	50	9.3 ± 3.0	65	9.3 ± 3.0	53	12.0 ± 3.0	66	11.5 ± 2.8
IgA	46	0.6 ± 0.6	63	0.4 ± 0.2	50	0.7 ± 0.4	65	0.6 ± 0.2*	53	0.8 ± 0.3	66	0.7 ± 0.3*
IgM	46	1.4 ± 0.9	63	1.6 ± 0.9	50	1.7 ± 0.7	65	2.0 ± 0.9	53	1.9 ± 0.7	66	1.8 ± 0.7

[a]Numbers do not match those in Table 3 because of blood sampling refusal, insufficient amount of blood collected, or shipping problems. *Significantly different from the bottle-fed value; $p \leq 0.05$.

study, the risk of otitis during the first year of life was mainly associated with prenatal exposure to DDE and HCB; these compounds were not measured in the Dutch study. The Inuit population and the Dutch population differ greatly with regard to the source of OC exposure: sea mammal fat consumption is the major source in Inuit people (*8*), whereas in the Netherlands, dairy products and industrial oils used by the food industry in the preparation of various foodstuffs are the largest contributors (*42*). These dietary sources are likely to differ substantially in terms of both contaminant and nutrient contents, which may have an impact on the susceptibility to infections.

Of the 213 infants originally included in the study, only 118 (55.4%) were followed during the entire first year of life. This raises the possibility of selection bias in our study group. However, further analyses of our data revealed that similar proportions of breast-fed and bottle-fed newborns did not attend any follow-up examinations or attended either one, two, or all three examinations (data not shown). Also, within the breast-fed group the percentages of infants who attended all three visits did not differ according to tertiles of OC concentration in breast milk. Moreover, maternal age and weight and infant weight and gestation length were not statistically different in the infants who attended at least one examination and in those who did not attend any follow-up examination. Hence, infants who were lost from the cohort or who missed one or two clinical examinations during the first year of life appear similar to those who completed the study. Finally, statistical analyses were also performed using only data from newborns who attended all three of the follow-up visits and similar results were observed (data not shown).

Our results could have been influenced by confounding factors. Three major factors have been associated with acute otitis media in several studies and therefore constitute possible confounding factors (*35*): breast-feeding (protective factor), crowding (risk factor), and passive smoking (risk factor). In the present study, we did not collect information on crowding or smoking; however, neither of these was likely to be a confounding factor here. Crowding as encountered in day-care centers can facilitate the propagation of infectious diseases among children; however, there are few day-care centers or family day-care homes in Nunavik. Crowding may also be present in large families, but based on data collected from the Santé Québec Inuit Health Survey (*24*), which was conducted during 1992–1993 in Nunavik, no relationship was noted between the number of children at home and maternal OC plasma concentrations. Analysis of smoking data also obtained from the Santé Québec Inuit Health Survey (*24*) revealed no association between cigarette consumption (average number of cigarettes smoked per day) and OC plasma concentrations in Inuit women between 18 and 39 years of age (75% of the women in this age group were smokers). In the present study we had information on the duration of breast-feeding, a protective factor for the development of otitis media. Associations between prenatal OC exposure and the risk of otitis media were not significantly modified when we performed logistic regression analyses to control for breast-feeding duration and for two factors associated with maternal OC body burden: maternal age and the total duration of past breast-feeding periods.

Because of results suggesting the existence of a relationship between middle ear infection and prenatal OC exposure, a reduction of organochlorine body burden in Inuit women of reproductive age appears desirable. The reduction of organochlorine body burden in this Inuit population has been encouraged by promoting the consumption of traditional food items that are high in nutritive elements and low in contaminants [i.e., red char (*Salvelinus salvelinus*)]. Because of study protocol limitations, we were unable to determine the influence of postnatal OC exposure on the risk of infectious diseases in Inuit infants. Nonetheless, because the risk of infectious diseases in breast-fed infants was not higher as compared to that of bottle-fed infants, it indicates that global postnatal OC exposure does not increase the susceptibility of Inuit infants to infections. As a result, women were advised not to modify their breast-feeding habits because of the beneficial aspects of this practice for both the infant and the mother.

References and Notes

1. Barrie LA, Gregor D, Hargrave B, Lake R, Muir D, Shearer R, Tracey B, Bidleman T. Arctic contaminants: sources, occurrence and pathways. Sci Total Environ 122:1–74 (1992).
2. Thomas DJ, Tracey B, Marshall H, Norstrom RJ. Arctic terrestrial ecosystem contamination. Sci Total Environ 122:135–164 (1992).
3. Lockhart WL, Wagemann R, Tracey B, Sutherland D, Thomas DJ. Presence and implications of chemical contaminants in the freshwaters of the Canadian Arctic. Sci Total Environ 122:165–243 (1992).
4. Muir DCG, Wagemann R, Hargrave BT, Thomas DJ, Peakall DB, Norstrom RJ. Arctic marine ecosystem contamination. Sci Total Environ 122:75–134 (1992).
5. Norstrom RJ, Simon M, Muir DCG, Schweinsburg RE. Organochlorine contaminants in arctic marine food chains: identification, geographical distribution, and temporal trends in polar bears. Environ Sci Technol 22:1063–1071 (1988).
6. Dewailly É, Nantel A, Weber JP, Meyer F. High levels of PCBs in breast milk of Inuit women from Arctic Quebec. Bull Environ Contam Toxicol 43:641–646 (1989).
7. Dewailly É, Nantel A, Bruneau S, Laliberté C, Ferron L, Gingras S. Breast milk contamination by PCDDs, PCDFs and PCBs in Arctic Quebec: a preliminary assessment. Chemosphere 25:1245–1249 (1992).
8. Dewailly É, Ayotte P, Bruneau S, Laliberté C, Muir DCG, Norstrom RJ. Inuit exposure to organochlorines through the aquatic food chain in Arctic Québec. Environ Health Perspect 101:618–620 (1993).
9. Dewailly É, Ayotte P, Laliberté C, Weber J-P, Gingras S, Nantel A. Polychlorinated biphenyl (PCB) and dichlorodiphenyl (DDE) concentrations in the breast milk of women in Quebec. Am J Public Health 86:1241–1246 (1996).
10. Ayotte P, Dewailly E, Ryan JJ, Bruneau S, Lebel G. PCBs and dioxin-like compounds in plasma of adult Inuit living in Nunavik (arctic Quebec). Chemosphere 34:1459–1468 (1997).
11. Safe SH. Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit Rev Toxicol 21:51–88 (1990).
12. Whitlock JP Jr. Mechanism of dioxin action: relevance to risk assessment. In: Biological Basis for Risk Assessment of Dioxins and Related Compounds (Gallo MA, Scheuplein RJ, Van Der Heijden KA, eds). Cold Spring Harbor, NY:Cold Spring Harbor Laboratory Press, 1991;351–366.
13. National Research Council. Biological Markers in Immunotoxicology. Washington, DC:National Academy Press, 1992;206.
14. Hoffman RE, Stehr-Green PA, Webb KB, Evans RG, Knutsen AP, Schramm WF, Staake JL, Gibson BB, Steinberg KK. Health effects of long-term exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. JAMA 255:2031–2038 (1986).
15. Vos JG, Luster MI. Immune alterations. In: Halogenated Biphenyls, Terphenyls, Napthalenes, Dibenzodioxins and Related Products (Kimbrough RD, Jensen AA, eds). New York:Elsevier Science Publishers, 1989;295–322.
16. Barnett JB, Barfield L, Walls R, Joyner R, Owens R, Soderberg LS. The effect of in utero exposure to hexachlorobenzene on the developing immune system of BALB/c mice. Toxicol Lett 39:263–274 (1987).
17. Holladay SD, Luster MI. Alterations in fetal thymic and liver hematopoietic cells as indicators of exposure to developmental immunotoxicants. Environ Health Perspect 104(suppl 4):809–813 (1996).
18. Chang KJ, Hsieh KH, Lee TP, Tang SY, Tung TC. Immunologic evaluation of patients with polychlorinated biphenyl poisoning: determination of lymphocyte subpopulations. Toxicol Appl Pharmacol 61:58–63 (1981).
19. Rogan WJ, Gladen BC, Hung KL, Koong SL, Shih LY, Taylor JS, Wu YC, Yang D, Ragan NB, Hsu CC. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. Science 241:334–336 (1988).
20. Chao WY, Hsu CC, Guo YLL. Middle-ear disease in children exposed prenatally to polychlorinated biphenyls and polychlorinated dibenzofurans. Arch Environ Health 52:257–262 (1997).
21. Duval B, Thérien F. Natalité, mortalité et morbidité chez les Inuit du Québec arctique. Rech Amérind Québec 12:41–50 (1982).
22. Dufour R. The otitis media among Inuit children. Proposal for a new approach. Arctic Med Res 47(suppl 1):659–665 (1988).
23. Proulx JF. Toward Healthy Communities on Hudson Coast. Povungnituk, Québec, Canada:Inuulitsivik Health Centre, 1988.
24. Santé Québec. A Health Profile of the Inuit: Report of the Santé Québec Health Survey Among the Inuit of Nunavik, 1992. Montréal, Québec, Canada:Ministère de la Santé et des Services Sociaux, Gouvernement du Québec, 1994.
25. Julien G, Baxter JD, Crago M, Ilecki HJ, Thérien F. Chronic otitis media and hearing deficit among native children of Kuujjuaraapik (Northern Quebec): a pilot project. Can J Public Health 78:57–62 (1987).
26. Ayukawa H. Hearing and Otitis Program on the Ungava Bay. Report on activities, January to July 1996. Kuujjuak, Québec, Canada:Tulatavik Hospital, 1996.
27. Lim DL, Demaria TF, Bakaletz LO. Current concepts of pathogenesis of otitis media: a review. Acta Otolaryngol suppl 458:174–180 (1988).
28. Jensen AA. Transfer of chemical contaminants into human milk. In: Chemicals Contaminants in Human Milk (Jensen AA, Slorach SA, eds). Boca Raton, FL:CRC Press, 1991;9–15.

29. Conacher HBS (Health Canada). Personal communication.
30. Ling D, McCoy RH, Levinson ED. The incidence of middle ear disease and its educational implications among Baffin Island Eskimo children. Can J Public Health 60:385–390 (1969).
31. Reed D, Struve S, Maynard JE. Otitis media and hearing deficiency among Eskimo children: a cohort study. Am J Public Health 57:1657–1662 (1967).
32. Ingvarsson L, Lundgren K, Stenstrom C. Occurrence of acute otitis media in children: cohort studies in an urban population. In: Workshop on Epidemiology of Otitis Media (Bluestone CD, Casselbrant ML, Scheetz MD, eds). Ann Otol Rhinol Laryngol 99(suppl 149):17–18 (1990).
33. Pukander J, Luotonen J, Sipilä M, Timonen M, Karma P. Incidence of acute otitis media. Acta Otolaryngol 93:447–453 (1982).
34. Teele DW, Klein JO, Rosner B. Epidemiology of otitis media during the first seven years of life in children in greater Boston: a prospective, cohort study. J Infect Dis 160:83–94 (1989).
35. Infante-Rivard C, Fernandez A. Otitis media in children: frequency, risk factors, and research avenues. Epidemiol Rev 15:444–465 (1993).
36. Timmermans FJ, Gerson S. Chronic granulomatous otitis media in bottle-fed Inuit children. Can J Med Assoc 122:545–547 (1980).
37. Dufour R. L'otite chez les enfants inuit: une question de mode alimentaire? Inuit Stud 8:77–87 (1984).
38. Abraham K, Knoll A, Ende M, Papke O, Helge H. Intake, fecal excretion, and body burden of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in breast-fed and formula-fed infants. Pediatr Res 40:671–679 (1996).
39. Ayotte P, Carrier G, Dewailly É. Health risk assessment for inuit newborns exposed to dioxin-like compounds through breast feeding. Chemosphere 32:531–542 (1996).
40. Haschke F, Male C, Pietschnig B. Infant exposure to PCDDs and PCDFs through breast milk and an approach to calculate body burden. Toxic Subst J 12:227–236 (1992).
41. Weisglas-Kuperus N, Sas TCJ, Koopman-Esseboom C, Van Der Zwan CW, De Ridder MAJ, Beishuizen A, Hooijkaas H, Sauer PJJ. Immunologic effects of background prenatal and postnatal exposure to dioxins and polychlorinated biphenyls in Dutch infants. Pediatr Res 38:404–410 (1995).
42. Huisman M, Eerenstein SE, Koopman-Esseboom C, Brouwer M, Fidler V, Muskiet FA, Sauer PJ, Boersma E. Perinatal exposure to polychlorinated biphenyls and dioxins through dietary intake. Chemosphere 31:4273–4287 (1995).